Pelvic Organ Prolapse

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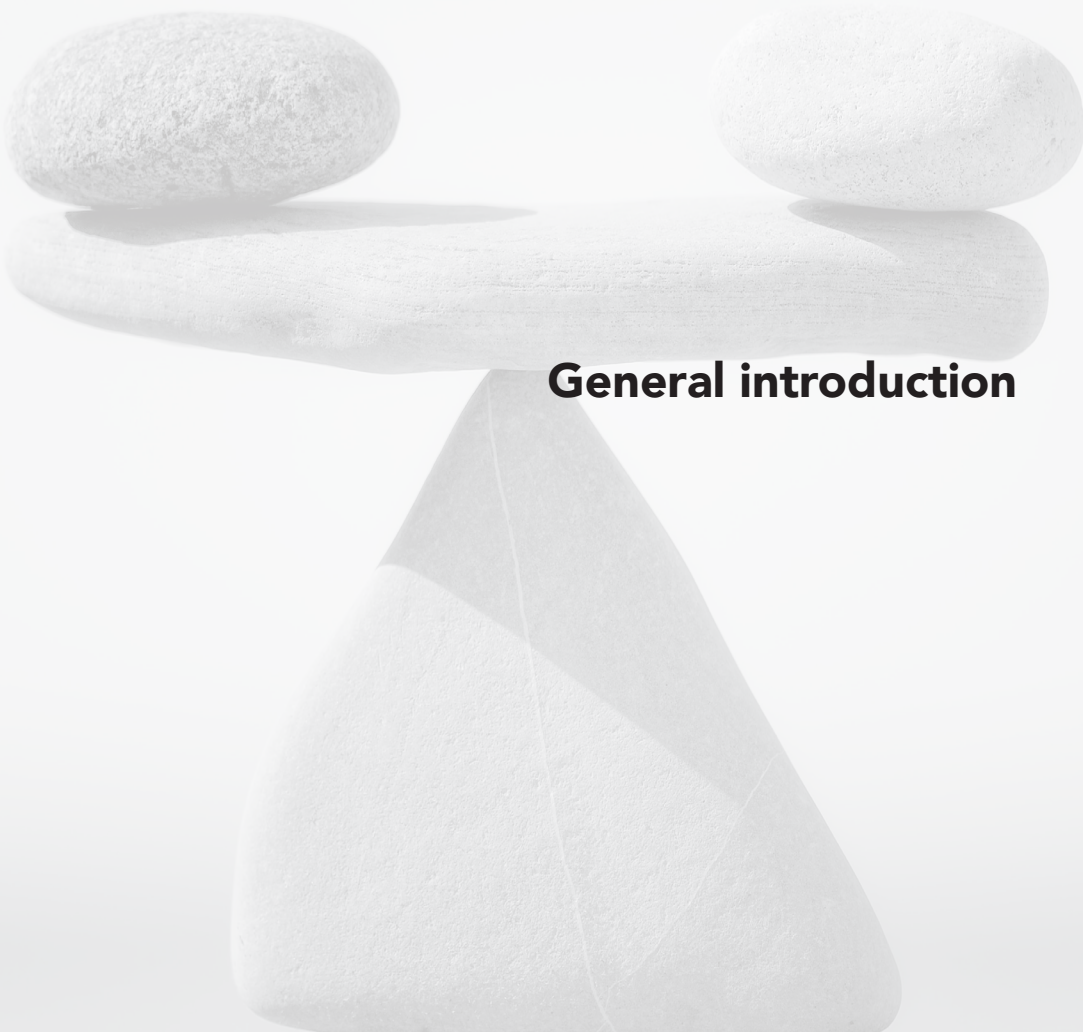

General introduction

PROLOGUE

Pelvic organ prolapse (POP) is a condition in women in which pelvic organs, such as the uterus, bladder or intestines, may push the vagina outside the body. This is due to pelvic floor muscle weakness and dysfunctional connective tissue. POP may cause chronic pelvic pain and pressure, urinary or fecal incontinence, sexual dysfunction and social isolation. This affects the quality of life of women with POP considerably.[2-4] Age is one of the important risk factors for POP.[5] With the worldwide ageing of the population, POP is a growing global health problem. The prevalence of POP in the Dutch population is 11%.[6] With approximately 13.000 (www.prismant.nl) surgical procedures each year, prolapse is the most common indication for gynecological surgery. This number is likely to increase approximately 35% over the next three decades, as the number of women over the age of 50 is expected to increase significantly.[7;8]

Native tissue repair is one of the common surgical therapies in POP. However, almost 30% of women operated for POP by this procedure will need a re-operation because of recurrent prolapse. In an attempt to improve this poor surgical outcome, synthetic implant materials were introduced in 1960 but are frequently used since the beginning of this century. Although the first results of these synthetic implant materials were promising, concerns arose about the safety of these new implants. Biological materials have not proven to be a realistic alternative. Although they have better tissue compatibility, and thus cause less local complications, the biological materials explored so far do not provide sufficient stability and support.

The urge for an alternative approach in pelvic reconstruction motivated us to start a multidisciplinary project with the aim to elucidate the fundamental problems in women with POP, and, possibly develop a therapy based on the principles of regenerative medicine. This thesis focuses on the necessary basic knowledge for this regenerative medicine approach in the pelvic floor and more specifically the anterior vaginal compartment.

BACKGROUND

Definition

Pelvic organ prolapse (POP) is defined as a descent of the female pelvic organs, including the bladder, uterus or vaginal vault after hysterectomy, or the small or large intestine, resulting in protrusion, bulging or herniation of one or more pelvic organs into or even out of the vagina.[9;10] POP is a result of mechanical failure in the pelvic floor tissue that supports the abdominal and pelvic organs. Pelvic organ support is maintained by a complex interaction between the levator ani muscles of the pelvic floor, the vagina and the supportive tissues of the vagina, such as ligaments and fascia. Traditionally, this structural support has been categorized in three distinct levels, highlighting support of the upper third of the vagina by the cardinal and uterosacral ligaments (level I), paravaginal attachments of the middle half of the vagina to the arcus tendineus fascia pelvis (level II), and the fusion of the lower third of the vagina to the perineal membrane and perineal body (level III; Figure 1). Endopelvic fascia include these ligaments and a looser areolar tissue that provides resilience and distensibility.[1]

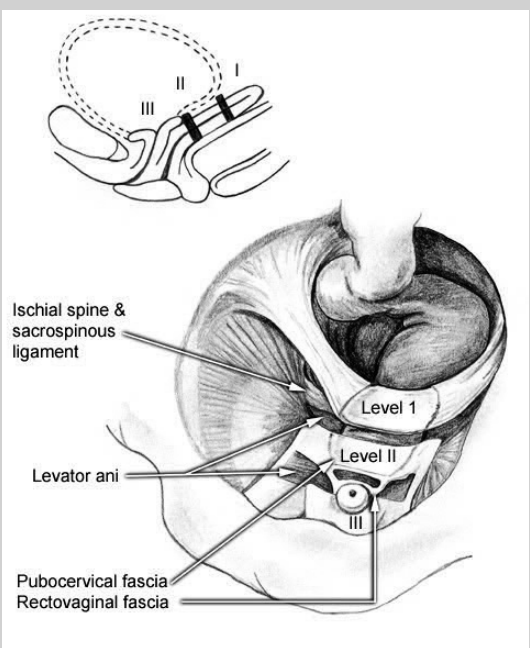

Figure 1. Levels of support.
Level 1: Suspension; the upper third of the vagina is suspended and supported by the cardinal–uterosacral ligaments and the connective tissue fibers of the upper paracolpium.
Level 2: Attachment; the middle third of the vagina is attached by the paracolpium (pubocervical fascia and recto vaginal fascia) laterally to the pelvic wall (arcus tendineus).
Level 3: Fusion: the lower third of the vagina fuses along the base of the urethra and the distal rectovaginal septum to the perineal body.
Source: DeLancey[1]

Depending on the defect at one or more levels, different types of prolapse can occur (Figure 2). The anterior compartment prolapse is the most common prolapse. It includes the descent of the bladder, the so-called cystocele.[11] Prolapse of the apical compartment is referred to as descensus uteri or, after hysterectomy, vaginal vault prolapse. Posterior vaginal wall prolapse involves the rectum (rectocele), but can also include the small and the large intestine. Since 1996, the Pelvic Organ Quantification System (POP-Q) is used to quantify the anatomic degree of pelvic organ prolapse in the three different compartments separately.[12] In the POP-Q system, prolapse can be divided in five different stages (stage 0 to stage IV). Stage 0 indicates normal anatomy with no prolapse and stage IV indicates complete eversion of the total length of the vagina (see addendum).

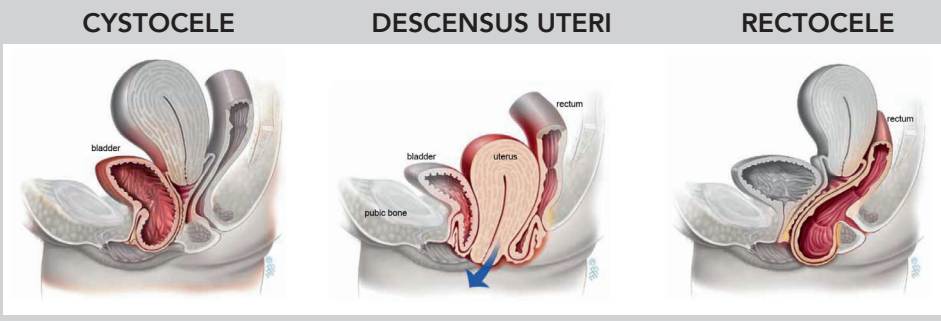

Figure 2. Pelvic organ prolapse in different compartments.
Source: International urogynecology Association (IUGA)

Pathophysiology and risk factors

The cause of POP is unknown, but the etiology of POP is considered to be multifactorial. Factors contributing to the weakening of the pelvic floor and the subsequent development of POP can be divided into genetic and acquired factors.[5;13] As an example of genetics, race predisposes a certain population of women to POP. African women, for example, are less prone to develop POP than Caucasian women. Inciting factors include pregnancy and parity as well as myopathy and neuropathy. Obesity, smoking, pulmonary disease and obstipation are examples of POP-promoting factors. Patients with these risk factors tend to develop POP in a higher frequency, with ageing and menopause as superimposing decompensating factors. Rather than one single factor, it is probable that combinations of anatomical, physiological, genetic, lifestyle, and reproductive factors interact throughout a woman's lifespan to contribute to mechanical failure of the pelvic floor causing pelvic floor dysfunction.[5]

There is growing evidence that genetic factors are indeed important in the development of prolapse. A recent systematic review on hereditary factors in POP demonstrated a substantially increased risk for POP in case of a positive family history[14], but the specific genetic defects have not been identified.[15-17] Based on a significant increase in prevalence of POP in genetic diseases of the connective tissue such as Ehlers–Danlos, Marfan syndrome, and cutis laxa, as well as in knock-out mice that lack extracellular matrix (ECM) molecules, a (genetic) connective tissue disorder is a likely etiological factor in POP.

Table 1. Risk factors involved in the development of pelvic organ prolapse.[13]

Predisposing	Inciting	Promoting	Decompensating
Genetics	Pregnancy	Obesity	Ageing
Race	Delivery	Smoking	Menopause
Gender	Myopathy	Pulmonary diseases	Neuropathy
	Neuropathy	Obstipation	Myopathy
	Pelvic surgery	Chronic lifting	General health

The anatomy on a tissue level

The connective tissue underlying the vagina is composed of cellular elements, fibroblasts, and smooth muscle cells (SMCs), surrounded by an extracellular matrix (ECM). Although fibroblasts are the main cells responsible for the synthesis and secretion of fibrillar components, also smooth muscle cells can synthesize these molecules. Collagen and elastin are the fundamental components that control the biomechanical properties of the vaginal tissue. Collagen fibers are very rigid and do not easily distort while elastic fibers provide elasticity and resilience. The proper function of connective tissues depends on the appropriate type, rate of synthesis, assembly, cross-linking and remodeling of the collagenous matrix.

The ECM is in a permanent state of remodeling. Its homeostasis depends on the balance between enzymatic synthesis and degradation by matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). Fibroblasts (FBs) are the cells responsible for maintaining extracellular matrix (ECM) homeostasis.[18] They produce molecules, and control anabolic and catabolic processes to remodel their surrounding matrix in response to mechanical and biochemical stimuli that originate from the ECM. These stimuli are recognized by transmembrane receptors, such as integrins. In this way there is a complex interaction between cells and the surrounding extracellular

matrix: the cell-matrix interaction. Changes on one level will affect the other, in order to adapt to changes in the environment.

During the course of a woman's life, the vaginal wall is one of the soft tissues that is constantly remodeled in reaction to the different forces that are applied to it. Pregnancy and parturition are the most extreme challenges in this respect. The weakening of the pelvic floor could thus be caused by an imbalance of its remodeling.[16;17] This imbalance might be caused by different functional characteristics of vaginal fibroblasts with changes in the concentration of MMPs and TIMPs, or changes in the collagen and elastin metabolism. Also, the quantity and quality of smooth muscle cells may play a crucial role.

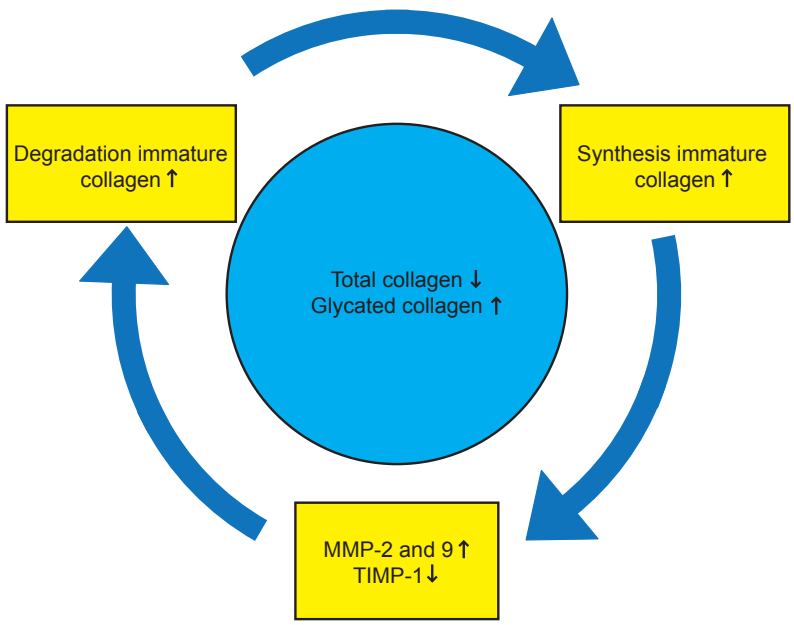

Figure 3. Collagen metabolism changes according to the theory of Jackson.[19]
Women with POP have a reduced total collagen content with an increased concentration of glycated collagen with results in brittle tissue with an impaired mechanical strenght. Also a relatively high content of immature collagen cross-links compared to non-POP patients is seen. Together with an increase in metalloproteinase (MMP) activity and a decrease in tissue inhibitors of metalloproteinases (TIMP), this newly formed collagen is degraded more easily than older glycated tissue. This results in an ongoing process of increased synthesis and degradation of newly formed collagen, leaving behind the old, glycated collagen.

One of the scarce studies on collagen metabolism in POP was published in 1996 by Jackson et al.[19] He found that increased collagenolytic activity by MMPs causes loss of collagen. Consequently, the synthesis of collagen is increased with immature collagen cross-links. This newly formed collagen is degraded more easily than older glycated collagen, resulting in a decrease of collagen content, and a relative increase in glycated collagen, resulting in tissue with an impaired mechanical strength and therefore more susceptible to rupture (Figure 3). Due to the complex cell-matrix interaction and the fact that genital prolapse develops over time, until now the causes and effects of the changes seen in the connective tissue in the supportive tissues of the pelvic floor have not been untwined.

Treatment

Treatment options for women with pelvic organ prolapse include observation, pelvic physiotherapy, the use of a pessary or surgery.

Non surgical treatment

Some women with advanced stages of prolapse have relatively few symptoms. If there is no sign of hydronephrosis due to chronic ureteral kinking or chronic bladder infections due to obstructive micturation, a watchful waiting policy can be an appropriate and reasonable treatment option.

Evidence for the efficacy of pelvic floor muscle training in the management of POP is so far minimal.[20] Daily pelvic floor muscle training can however slow the progression of anterior vaginal wall prolapse.[21] For those who do suffer from symptomatic POP, but do not opt for or are not able to have surgical treatment, pessary treatment can be considered. The use of a pessary is a patient friendly, minimal invasive and safe treatment. It is able to adequately relieve many symptoms of prolapse and may contribute to improvement of quality of life scores in women suffering from POP.[22]

Surgical treatment

The surgical treatment of prolapse is generally focused on restoring the support of the vagina by connective tissue. Depending on the type of prolapse, a different operation or combination of different procedures is indicated. In most countries the conventional native tissue repair is used to treat a cystocele or rectocele, called: anterior or posterior colporrhaphy or fascia plication (Figure 4). During this procedure, the redundant tissue of the pubocervical fascia (anterior) or the rectovaginal septum (posterior) is plicated in the midline with absorbable sutures. With this type of primary POP surgery the risk of a recurrence or re-operation is 29%, mostly within two years after surgery.[23;24]

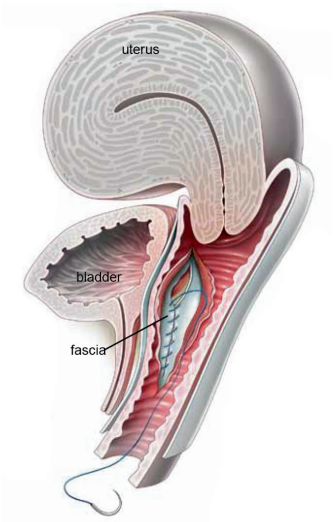

Figure 4. Anterior colporrhaphy.
Source: International Urogynecology Association (IUGA)

In an attempt to improve surgical outcomes, synthetic and biological implants have been introduced in reconstructive pelvic surgery.[25] Primary permanent synthetic implants, as well as absorbable synthetic implants or biological products derived from human cadavers (allografts) or animals (xenografts) have been introduced on the market. However, evidence of efficacy of these products is lacking[26] and complication rates are high.[27-31]

Absorbable synthetic and biological implants appear to be less harmful due to an easy incorporation into native tissue. Their long term efficacy as well as their possible adverse effects are unclear. There is some evidence that absorbable mesh materials over time do not generate sufficiently strong new tissue, decreasing the durability of reconstructive pelvic surgery with these types of implants.[32;33] At this moment, permanent synthetic meshes made of polypropylene dominate the market. Despite attempts to increase the biocompatibility for these products[34], complications such as erosions, pain, infections, and vaginal shrinkage still exist.[30;31;34]

Considering this, the US Food and Drug Administration have recently published a public health notification on the serious complications associated with synthetic mesh use for POP procedures (http://www.fda.gov/ medicaldevices/safety/ alertsandnotices/ publichealthnotifications/ucm061976.htm). In reaction to this FDA warning, the International UroGynecology Association (IUGA) has formulated a consensus document for optimizing the safety and appropriateness of mesh use

in pelvic reconstructive surgery.[35] This has been adopted by the Dutch society of pelvic floor with the publication of '*Nota gebruik van kunststof materiaal bij prolaps chirurgie*' version 2.0 (http://nvog-documenten.nl/index.php?pagina=/richtlijn/pagina.php &fSelectNTG_110=111&fSelectedSub=110).

Regenerative medicine, tissue engineering and cell based therapy

It goes without saying that new concepts are urgently needed. Tissue engineering is an emerging field in regenerative medicine that could provide attractive alternatives, alone or as an adjunct to surgical reconstructive surgery for pelvic organ prolapse.[36-39]

Regenerative medicine is an interdisciplinary field that aims at replacing or regenerating human cells, tissues or organs to restore or establish normal function. The classical tissue engineering approach consists of the application of a scaffold, seeded with (stem)cells and growth factors. A variety of biomedical approaches, such as the use of stem or progenitor cells (cell based therapies), regeneration induction by biologically active molecules or transplantation of in vitro grown organs and tissues is used.[36] The cells involved are preferably autologous to reduce the chances of any immunological reaction. This does, of course, increase the risk of reintroducing a possible genetic cause of the prolapse. The cells can be freshly isolated or cultured in vitro for injection purposes. Cell based injection therapy in urogynecology has focused on the regeneration of the urethral sphincter for the treatment of stress urinary incontinence (SUI).[40,41] Animal studies demonstrated that cells (autologous muscle derived stem or progenitor cells) cultured in vitro survive the injection in the urethral sphincter. Also a repair process resembling the normal regenerative process in skeletal muscles is initiated. In clinical studies with using cell based injection therapy patients with SUI are cured in 20-50% of the cases. Only minor complications were observed.[40;41] In the treatment for POP, a simple injection of cells to regenerate damaged vaginal tissue is not feasible without anchoring to a biodegradable scaffold that will provide temporary mechanical support to the weakened supportive tissue of the pelvic floor.

Tissue Engineering Approach in Pelvic Organ Prolapse

The TEAPOP project (Tissue Engineering Approach in Pelvic Organ Prolapse) started in 2008 and aims at the systematic exploration of the feasibility of applying regenerative medicine concepts to pelvic organ prolapse. The ultimate goal of this project is to develop a new method to successfully repair the fascial tissues in a one-step surgical approach by using a combination of a highly biocompatible and biodegradable scaffold, providing sufficient tensile strength during the replacement of the scaffold, by patient's own connective tissue, with regeneration-component

(stem) cells or inductive growth factors. To achieve this goal we first need to obtain more insight into the underlying pathophysiology of pelvic organ prolapse. We therefore performed a multiparameter study (COLPOP study: collagen metabolism in patients with and without Pelvic Organ Prolapse) focusing on anterior vaginal wall tissue (Figure 5).

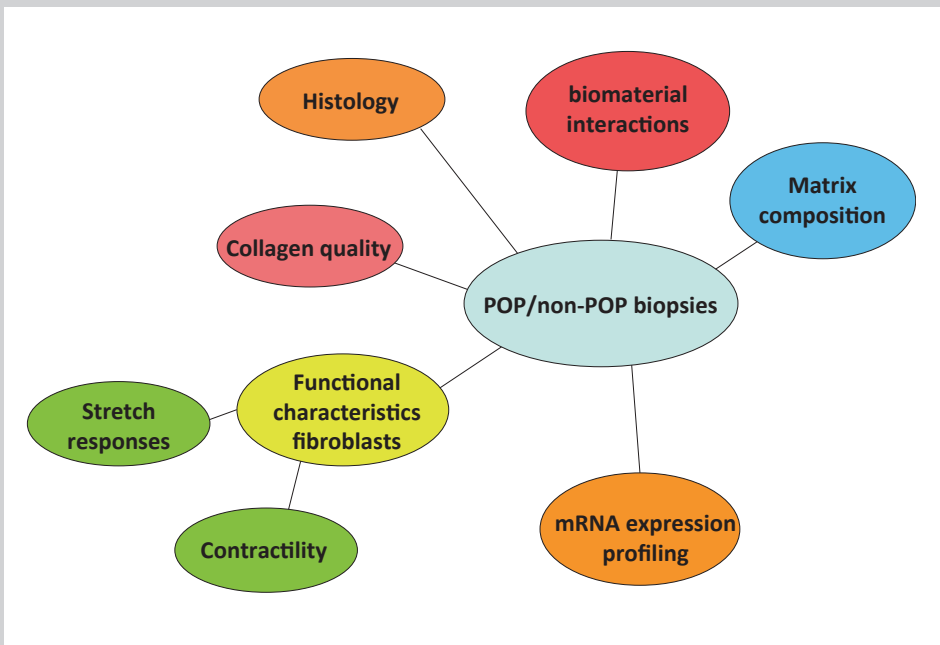

Figure 5. Multiparameter study on anterior vaginal wall tissue of women with a cystocele.

OUTLINE OF THE THESIS

The aim of this thesis is to assess to what extent POP is an intrinsic or an acquired disease. We need to know whether the relationship between POP and the changes in the connective tissue is causal and if so, in what direction. In other words: does excessive tissue stretching at the prolapse site lead to aberrant extracellular matrix metabolism or is aberrant extracellular matrix metabolism the cause of prolapse. This basic understanding is essential when one seeks to develop new therapeutic strategies in POP.

Moreover, if (stem) cells are used to contribute to tissue regeneration by proliferation and differentiation into (myo) fibroblasts and by formation of the adequate connective tissue, we need to know which cells are appropriate for the preparation

of a bio-engineered construct. Therefore, the second aim is to assess the functional characteristics of fibroblasts of women with prolapse. If POP is largely an acquired disease, knowledge of the functional characteristics of fibroblasts will provide information about the possibility to reverse or halt the process of deterioration of the cells and the extracellular matrix. This will help us to determine which kind of strategies can be used in reconstruction of the pelvic floor.

In this thesis the following specific objectives are addressed:

- To provide a systematic review of literature regarding changes in connective tissue in patients with pelvic organ prolapse. (Chapter 2)

- To identify possible intrinsic and acquired effects in connective tissues of pelvic organ prolapse by comparing (immuno-) histological and biochemical features of the (normal) precervical anterior vaginal wall and the prolapsed anterior vaginal wall of women with pelvic organ prolapse. (Chapter 3)

- To identify prolapse related dysregulated pathways involved in extracellular matrix metabolism by using micro-array technology. (Chapter 4)

- To study the heterogeneity of women with prolapse at a molecular level. (Chapter 4)

- To investigate the functional characteristics of vaginal fibroblasts derived from women with pelvic organ prolapse compared to healthy vaginal fibroblasts in vitro. (Chapter 5 and chapter 6)

In chapter 2 we provide an overview of our current understanding of changes in pelvic floor connective tissue in women with POP. In chapter 3 we address the question whether the relationship between POP and collagen metabolism is causal, i.e. that excessive tissue stretching at the prolapse site leads to changes in tissue composition. Tissue samples taken from the POP-site and the non-POP site of the vagina in the same POP patient are compared. In addition, comparisons are made with similar samples from non-POP controls. These data will encompass the (immuno-)histological and biochemical characteristics of the specimens. The (immuno-)histochemical evaluation of the tissue will provide information about the structure and composition of the vaginal tissue(s) and the possible differences between prolapsed and not prolapsed sites within POP patients, or between patients and healthy controls. The biochemical analysis will shed light, among

others, on the amount and quality of the extracellular matrix per se and its building blocks (collagens and non-collagenous proteins).

With whole genome micro-array analysis, we seek to identify prolapse related disregulated pathways by comparing gene expression profiles of prolapsed and non-prolapsed anterior vaginal wall tissue within the same patient (intra-patient comparison). As the population of women with prolapse is characterized by its heterogeneity we additionally study this heterogeneity at the molecular level by comparing the tissue gene expression profiles in the non-prolapsed anterior vaginal wall of these POP women. The findings are described in chapter 4.

Moreover, using primary cultures, we will determine whether fibroblasts derived from prolapsed and non-prolapsed tissues of patients differ in their functional characteristics in vitro. In chapter 5 the effects of cyclic mechanical loading on the gene expression and protein levels of ECM remodeling factors are evaluated in a pilot study of women with different degrees of prolapse. Also we evaluate whether the enzymatic activity of remodeling factors is affected by the presence of artificial polymeric substrates. In chapter 6 we repeat the experiments described in chapter 5 with fibroblasts from the COLPOP trial and expand the experiment with evaluations of the contractile capacity of fibroblasts. Also the phenotype of the cells and the proliferation rate of the cells are determined.

Finally, in chapter 7 the information gathered in this thesis is integrated into a general discussion on the changes seen in the extracellular matrix and the functionality of the cells in this matrix of prolapsed vaginal wall tissue.

REFERENCE LIST

1. Delancey JO. Anatomic aspects of vaginal eversion after hysterectomy. Am.J.Obstet.Gynecol. 1992;166:1717-24.
2. Delancey JO. Anatomy and biomechanics of genital prolapse. Clin.Obstet.Gynecol. 1993;36:897-909.
3. Norton PA. Pelvic floor disorders: the role of fascia and ligaments. Clin.Obstet.Gynecol. 1993;36:926-38.
4. Jelovsek JE, Maher C, Barber MD. Pelvic organ prolapse. Lancet 2007;369:1027-38.
5. Delancey JO, Kane LL, Miller JM, Patel DA, Tumbarello JA. Graphic integration of causal factors of pelvic floor disorders: an integrated life span model. Am.J.Obstet.Gynecol. 2008;199:610-15.
6. Slieker-ten Hove MCP, Pool-Goudzwaard AL, Eijkemans MJC, Steegers-Theunissen RPM, Burger CW, Vierhout ME. Symptomatic pelvic organ prolapse and possible risk factors in a general population. Am.J.Obstet.Gynecol. 2009;200:184-87.
7. Kirby AC, Luber KM, Menefee SA. An update on the current and future demand for care of pelvic floor disorders in the United States. Am.J.Obstet.Gynecol. 2013;209:584-85.
8. Boyles SH, Weber AM, Meyn L. Procedures for pelvic organ prolapse in the United States, 1979-1997. Am.J.Obstet.Gynecol. 2003;188:108-15.
9. Jelovsek JE, Maher C, Barber MD. Pelvic organ prolapse. Lancet 2007;369:1027-38.
10. Haylen BT, De Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Int.Urogynecol.J. 2010;21:5-26.
11. Hendrix SL, Clark A, Nygaard I, Aragaki A, Barnabei V, McTiernan A. Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. Am.J.Obstet.Gynecol. 2002;186:1160-66.
12. Bump RC, Mattiasson A, Bo K, Brubaker LP, Delancey JO, Klarskov P et al. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. Am.J.Obstet.Gynecol. 1996;175:10-17:10-17.
13. Bump RC, Norton PA. Epidemiology and natural history of pelvic floor dysfunction. Obstet.Gynecol.Clin.North Am. 1998;25:723-46.
14. Lince SL, van Kempen LC, Vierhout ME, Kluivers KB. A systematic review of clinical studies on hereditary factors in pelvic organ prolapse. Int.Urogynecol.J. 2012;23:1327-36.
15. Cartwright R, Mangera A, Tikkinen KAO, Chapple C. What was hot at the ICS meeting Glasgow, Scotland, 2011. Neurourol.Urodyn. 2012;31:2-6.
16. Mosier E, Lin VK, Zimmern P. Extracellular matrix expression of human prolapsed vaginal wall. Neurourol.Urodyn. 2010;29:582-86.
17. Bortolini MAT, Rizk DEE. Genetics of pelvic organ prolapse: crossing the bridge between bench and bedside in urogynecologic research. Int.Urogynecol.J. 2011;22:1211-19.
18. Meyer S, Achtari C, Hohlfeld P, Juillerat-Jeanneret L. The contractile properties of vaginal myofibroblasts: is the myofibroblasts contraction force test a valuable indication of future prolapse development? Int.Urogynecol.J.Pelvic.Floor.Dysfunct. 2008;19:1399-403.
19. Jackson SR, Avery NC, Tarlton JF, Eckford SD, Abrams P, Bailey AJ. Changes in metabolism of collagen in genitourinary prolapse. Lancet 1996;347:1658-61.
20. Rosenbaum TY. Pelvic floor physiotherapy for women with urogenital dysfunction: indications and methods. Minerva Urol.Nefrol. 2011;63:101-07.
21. Piya-Anant M, Therasakvichya S, Leelaphatanadit C, Techatrisak K. Integrated health research program for the Thai elderly: prevalence of genital prolapse and effectiveness of pelvic floor exercise to prevent worsening of genital prolapse in elderly women. J.Med.Assoc.Thai. 2003;86:509-15.
22. Lamers BHC, Broekman BMW, Milani AL. Pessary treatment for pelvic organ prolapse and health-

related quality of life: a review. Int.Urogynecol.J. 2011;22:637-44.
23. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet.Gynecol. 1997;89:501-06.
24. Denman MA, Gregory WT, Boyles SH, Smith V, Edwards SR, Clark AL. Reoperation 10 years after surgically managed pelvic organ prolapse and urinary incontinence. Am.J.Obstet.Gynecol. 2008;198:555.
25. Silva WA, Karram MM. Scientific basis for use of grafts during vaginal reconstructive procedures. Curr.Opin.Obstet.Gynecol. 2005;17:519-29.
26. Maher CM, Feiner B, Baessler K, Glazener CMA. Surgical management of pelvic organ prolapse in women: the updated summary version Cochrane review. Int.Urogynecol.J. 2011;22:1445-57.
27. Milani ALF, Vollebregt A, Roovers JP, Withagen MIJ. [The use of mesh in vaginal prolapse]. Ned.Tijdschr.Geneeskd. 2013;157:A6324.
28. Vollebregt A, Fischer K, Gietelink D, van der Vaart CH. Effects of vaginal prolapse surgery on sexuality in women and men; results from a RCT on repair with and without mesh. J.Sex Med. 2012;9:1200-11.
29. Withagen MI, Vierhout ME, Hendriks JC, Kluivers KB, Milani AL. Risk factors for exposure, pain, and dyspareunia after tension-free vaginal mesh procedure. Obstet.Gynecol. 2011;118:629-36.
30. Sung VW, Rogers RG, Schaffer JI, Balk EM, Uhlig K, Lau J et al. Graft use in transvaginal pelvic organ prolapse repair: a systematic review. Obstet.Gynecol. 2008;112:1131-42.
31. Abed H, Rahn DD, Lowenstein L, Balk EM, Clemons JL, Rogers RG. Incidence and management of graft erosion, wound granulation, and dyspareunia following vaginal prolapse repair with graft materials: a systematic review. Int.Urogynecol.J. 2011;22:789-98.
32. Claerhout F, Verbist G, Verbeken E, Konstantinovic M, De Ridder D, Deprest J. Fate of collagen-based implants used in pelvic floor surgery: a 2-year follow-up study in a rabbit model. Am.J.Obstet.Gynecol. 2008;198:94-96.
33. Menefee SA, Dyer KY, Lukacz ES, Simsiman AJ, Luber KM, Nguyen JN. Colporrhaphy compared with mesh or graft-reinforced vaginal paravaginal repair for anterior vaginal wall prolapse: a randomized controlled trial. Obstet.Gynecol. 2011;118:1337-44.
34. Patel H, Ostergard DR, Sternschuss G. Polypropylene mesh and the host response. Int.Urogynecol.J. 2012;23:669-79.
35. Davila GW, Baessler K, Cosson M, Cardozo L. Selection of patients in whom vaginal graft use may be appropriate. Consensus of the 2nd IUGA Grafts Roundtable: optimizing safety and appropriateness of graft use in transvaginal pelvic reconstructive surgery. Int.Urogynecol.J. 2012;23 Suppl 1:S7-14.
36. Boennelycke M, Gras S, Lose G. Tissue engineering as a potential alternative or adjunct to surgical reconstruction in treating pelvic organ prolapse. Int.Urogynecol.J. 2013;24:741-47.
37. Olson JL, Atala A, Yoo JJ. Tissue engineering: current strategies and future directions. Chonnam.Med.J. 2011;47:1-13.
38. Demirbag B, Huri PY, Kose GT, Buyuksungur A, Hasirci V. Advanced cell therapies with and without scaffolds. Biotechnol.J. 2011;6:1437-53.
39. Aboushwareb T, McKenzie P, Wezel F, Southgate J, Badlani G. Is tissue engineering and biomaterials the future for lower urinary tract dysfunction (LUTD)/pelvic organ prolapse (POP)? Neurourol.Urodyn. 2011;30:775-82.
40. Wang HJ, Chuang YC, Chancellor MB. Development of cellular therapy for the treatment of stress urinary incontinence. Int.Urogynecol.J. 2011;22:1075-83.
41. Gras S, Lose G. The clinical relevance of cell-based therapy for the treatment of stress urinary incontinence. Acta Obstet.Gynecol.Scand. 2011;90:815-24.